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PATENT COOPERATION TREATY

PCT

REC'D 25 OCT 2000

WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
NCB/P21046WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/01828	09/06/1999	09/06/1998
International Patent Classification (IPC) or national classification and IPC G01N33/50		
Applicant QUEEN MARY & WESTFIELD COLLEGE et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☒ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05/01/2000	23.10.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Cuendet, P Telephone No. +49 89 2399 8690

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01828

I. Basis f th rep rt

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-20 as originally filed

Claims, No.:

1-17 as originally filed

Drawings, sheets:

1/7-7/7 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

☐ restricted the claims.

☐ paid additional fees.

☐ paid additional fees under protest.

☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	2,6-10,12-15,17
	No:	Claims	1,3-5,11,16
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-17
Industrial applicability (IA)	Yes:	Claims	11-17
	No:	Claims	

2. Citations and explanations

   see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

1). **Preamble**

Concept: the present method comprises, assaying a blood sample for 2 markers (free β-hCG and Inhibin A) and the use of the 2 measure levels of the markers to predict the risk of pre-eclampsia.

Comments: in the light of the present description hCG is an alternative to "free β-hCG"; cf. bottom of p.3. To measure the 2 markers "commercially available kits" can be used; cf. present pp. 3 and 8.

2). **Point IV.**

The subject-matter of claims 11-15 (apparatus) cannot be related to the concept of the present method*: the claimed apparatus (claim 11) is not for assaying a blood sample.

---

*) see in item 1 above

3). **Point V.2.**

3.1. According to **D1**: WO-A-98/02751, which is considered to be the closest prior art, it was known that the concentrations of Inhibin A as well as of hCG can be used for predictive diagnosis of pre-enclampsia, cf. D1, p.3; Fig.2; claim 5; the corresponding hormone assays are indicated on p.7 of D1; the method of D1 is, apparently, carried out after 20 weeks of pregnacy (Fig.4). Thus, in the light of the above/of p.3 of the present description, the subject-matter of claims 1, 3-5 and 16 is lacking novelty or at least an inventive step (for claims 1, 3-10 and 16) regarding D1.

3.2. For one skilled in the art, the last paragraph on p.10 of D1 implies the use of a computer, i.e. of an apparatus having "data input means" and "calculation means" as claimed in present claim 11. One skilled in the art knows that computers were available on the market before the relevant date. Thus, the subject-matter of claims 11 is lacking novelty regarding D1 and/or an inventive step (for claims 11-15) regarding the common knowledge of one skilled in the art.

3.3. The present application does not demonstrate that the method/kit according

to claim 2/claim 17 would give rise to unexpected effects compared to the method according to D1. Thus, the subject-matter of claims 2 and 17 would appear to lack an inventive step regarding D1.

3.4. Claims 1-10 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT; cf. **claim 1(a)**. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

4). **Point VIII.**
The subject-matter of claim 16 is vague and indefinite: the feature "means for assaying..." is defined according to the results to be achieved and not by the (essential) products of that kit; for these "(essential) produchts" see (i) the last comment in above items 1 and (ii) item 3.1.

5). **Point VII.**
Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 is not mentioned in the description, nor is this document identified therein.

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Articl 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
NCB/P21046WO		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/01828	09/06/1999	09/06/1998
Applicant		
QUEEN MARY & WESTFIELD COLLEGE et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   [ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   [ ] contained in the international application in written form.

   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

   [ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 99/01828

A. CLASSIFICATION OF SUBJECT MATTER

G01N33/76, G01N33/74, G01N33/68

According to International Patent Classification (IPC) or to both national classification and IPC 6

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

G01N, C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98/02751 A (ISIS INNOVATION LIMITED et al.) 22 January 1998, abstract, claims. --	1,3-5, 16
A	CHEMICAL ABSTRACTS, vol. 127, no. 3, 21 July 1997 Columbus, Ohio, US, abstract no. 32396r, MUTTUKRISHNA, S. ET AL.: "Activin A and inhibin A as possible endocrine markers for pre-eclampsia", page 467, column 1; & Lancet 1997, 349(9061), pages 1285-1288 (Eng). --	1,3-5
A	US 5712103 A (LEAVITT et al.) 27 January	1

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 September 1999	21.12.99

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	SCHNASS e.h.

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	1998, abstract.	
A	CHEMICAL ABSTRACTS, vol. 123, no. 13, 25 September 1995 Columbus, Ohio, US, abstract no. 166819g, MARINOFF, D.N. ET AL.: "Utility of a single plasma fibronectin level for the prediction of preeclampsia", page 799, column 2; & J. Matern.-Fetal Med. 1995, 4(4), pages 160-165 (Eng).	1
A	CHEMICAL ABSTRACTS, vol. 126, no. 1, 06 January 1997 Columbus, Ohio, US, abstract no. 4166b, KYLE, P.M. ET AL.: "A comparision of the inactive urinary kallikrein:Creatinine ratio and the angiotensin angiotensin sensitivity test for the prediction of pre-eclampsia", page 474, columns 1-2; & Br. J. Obstet. Gynaecol. 1996, 103(10), pages 981-987 (Eng).	1
A	WO 90/07122 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 28 June 1990, abstract, claims.	1
A,P	WO 98/28006 A (CAMBRIDGE UNIVERSITY TECHNICAL SERVICES LIMITED) 02 July 1998, page 3, lines 13-23.	1
A,P	CHEMICAL ABSTRACTS, vol. 129, no. 3, 20 July 1998 Columbus, Ohio, US, abstract no. 26642t, BOFFA, M.-C. ET AL.: "Predictive value of plasma thrombomodulin in pre-eclampsia and gestational hypertension", page 494, column 1;	1

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& Thromb. Haemostasis 1998, 79(6), pages 1092-1095 (Eng). --	
A,P	CHEMICAL ABSTRACTS, vol. 129, no. 15, 12 October 1998 Columbus, Ohio, US, abstract no. 187926d, PEREZ-BLANCO, F. J. ET AL.: "Urinary N-acetyl-beta-glucosaminidase in the prediction of preeclampsia", page 515, column 1; & Clin. Nephrol. 1998, 50(3), pages 169-171 (Eng). --	1
A,P	DATABASE WPI Week 9851, Derwent Publications Ltd., London, GB; Class B04, AN 98-607817; & RU 2107916 C (IVAN MOTHERS CHILDREN RES INST), abstract. ----	1

ANHANG

zum internationalen Recherchenbericht über die internationale Patentanmeldung Nr,

ANNEX

to the International Search Report to the International Patent Application No.

ANNEXE

au rapport de recherche international relatif à la demande de brevet international n°

PCT/GB 99/01828 SAE 237432

In diesem Anhang sind die Mitglieder der Patentfamilien der im obengenannten internationalen Recherchenbericht angeführten Patentdokumente angegeben. Diese Angaben dienen nur zur Unterrichtung und erfolgen ohne Gewähr.

This Annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The Office is in no way liable for these particulars which are given merely for the purpose of information.

La présente annexe indique les membres de la famille de brevets relatifs aux documents de brevets cités dans le rapport de recherche international visée ci-dessus. Les renseignements fournis sont donnés à titre indicatif et n'engagent pas la responsibilité de l'Office.

Im Recherchenbericht angeführtes Patentdokument Patent document cited in search report Document de brevet cité dans le rapport de recherche	Datum der Veröffentlichung Publication date Date de publication	Mitglied(er) der Patentfamilie Patent family member(s) Membre(s) de la famille de brevets	Datum der Veröffentlichung Publication date Date de publication
WO A1 9802751	22-01-1998	EP A1 927355 GB A0 9614615	07-07-1999 04-09-1996
US A 5712103	27-01-1998	keine - none - rien	
WO A1 9007122	28-06-1990	EP A1 440750 EP A4 440750 JP T2 4505804 US A 5238819	14-08-1991 09-10-1991 08-10-1992 24-08-1993
WO A1 9828006	02-07-1998	AU A1 53312/98 GB A0 9626702	17-07-1998 12-02-1997
RU C 2107916		keine - none - rien	

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

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NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 03 February 2000 (03.02.00)	
International application No. PCT/GB99/01828	**Applicant's or agent's file reference** NCB/P21046WO
International filing date (day/month/year) 09 June 1999 (09.06.99)	**Priority date** (day/month/year) 09 June 1998 (09.06.98)
Applicant WALD, Nicholas, John et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

05 January 2000 (05.01.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Olivia RANAIVOJAONA
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PCT GP

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
G01N 33/50

A2

(11) International Publication Number: WO 99/64860

(43) International Publication Date: 16 December 1999 (16.12.99)

(21) International Application Number: PCT/GB99/01828

(22) International Filing Date: 9 June 1999 (09.06.99)

(30) Priority Data:
9812432.4 9 June 1998 (09.06.98) GB

(71) Applicants *(for all designated States except US)*: QUEEN MARY & WESTFIELD COLLEGE [GB/GB]; Mile End Road, London E1 4NS (GB). UNIVERSITY OF OXFORD [GB/GB]; Wellington Square, Oxford OX1 2JD (GB).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WALD, Nicholas, John [GB/GB]; Department of Environmental and Preventive Medicine, St Bartholomew's Hospital, Queen Mary & Westfield College, Charterhouse Square, London EC1M 6BQ (GB). REDMAN, Christopher [GB/GB]; Nuffield Department of Obstetrics and Gynaecology, University of Oxford, John Radcliffe Hospital, Headington, Oxford OX3 9DU (GB).

(74) Agents: SHEARD, Andrew, Gregory et al.; Kilburn & Strode, 20 Red Lion Street, London WC1R 4PJ (GB).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

Without international search report and to be republished upon receipt of that report.

(54) Title: PREDICTIVE TEST FOR PRE–ECLAMPSIA

(57) Abstract

A method is provided which enables a prediction to be made about the risk of a pregnant woman developing pre–eclampsia which comprises an analysis of the serum levels of screening markers, Inhibin A and free β–hCG. Apparatus for carrying out the determination of the risk of developing pre–eclampsia based on the analysis of the serum samples is also provided.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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EE	Estonia	LR	Liberia	SG	Singapore		

PREDICTIVE TEST FOR PRE-ECLAMPSIA

The present invention relates to a test which can be used to predict pre-eclampsia in pregnant women.

Pre-eclampsia is a disorder of human pregnancy which affects around 5 to 10% of pregnancies. The underlying cause of pre-eclampsia remains unclear in spite of extensive clinical and basic research. Pre-eclampsia is the definition given to the condition in pregnancy in which elevated blood pressure is associated with proteinuria. Pre-eclampsia is distinct from eclampsia which is additionally associated with convulsions. Pre-eclampsia is defined in Souhami & Moxham *Textbook of Medicine*, Second edition, Churchill Livingstone (1994), as an abnormal rise in blood pressure between the first and second halves of pregnancy of $\geq$ 30/20 mmHg, with abnormal urate levels of >0.35 mmol/l at 32 weeks or >0.4mmol/l thereafter, associated with proteinuria, impaired renal function and clotting disorders. The consequences of pre-eclampsia are serious and include reduced uteroplacental perfusion, foetal growth retardation, pre-term birth, and increased foetal and maternal morbidity and mortality.

There have been many attempts to provide a reliable predictive test for pre-eclampsia. Previous suggestions have involved assays for the levels of circulating biochemical markers in the mother's blood but to date the scientific literature on this issue is contradictory and inconclusive. The following hormones have all been identified as possible markers in an elevation of levels might be predictive of pre-eclampsia in maternal plasma: progesterone, oestradiol, total human chorionic gonadotrophin (hCG), corticotrophin-releasing factor (CRF), adrenocorticotrophin (Muller *et al Am. J. Obst. Gynecol.* **175** 37-40 (1996); Ashour *et al Am. J. Obst. Gynecol.* **176** 438-444 (1997); Hsu *et al Am. J. Obst. Gynecol.* **170** 1135-1138 (1994); Wenstrom *et al A. J. Obst. Gynecol.* **171** 1038-1041 (1994)). Conversely, levels of oestriol, human placental lactogen and cortisol are unchanged or decreased. Whilst circulating CRF has been proposed as a prognostic marker for pre-eclampsia, treatment of hypertension does not

influence maternal CRF levels and nor has any correlation been found between CRF levels and mean blood pressure.

Other possible markers which have been suggested are Activin A and Inhibin A. Activin is a hypophysiotrophic factor produced by the placenta which is know to act as a growth factor having activity in modulating cell growth and differentiation. Currently, there are three forms of activin which are recognised to exist as homodimeric proteins: Activin A ($\beta_A\beta_A$), Activin AB ($\beta_A\beta_B$) and Activin B ($\beta_B\beta_B$) in which the subunits are linked by disulphide bridges. Inhibins are heterodimeric proteins consisting of $\alpha\beta_A$ (Inhibin A) and $\alpha\beta_B$ (Inhibin B) subunits also linked by disulphide bridges. Additionally monomeric Inhibin α subunits are present in the circulation and follicular fluid. Inhibin is thought to have an endocrine role which inhibits pituitary production of follicle-stimulating hormone (FSH). Muttikrishna *et al* (*The Lancet* **349** 1285-1288 (1997)) have proposed that Activin A and Inhibin A might be suitable markers for the onset of pre-eclampsia. These proteins were suggested because they were thought to be more sensitive markers than hCG or corticotrophin-releasing hormone where there is a considerable overlap in elevated hormone levels between control and pre-eclamptic women.

However, it has now been found that a predictive test for pre-eclampsia which is based on levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A can in fact provide a surprisingly improved level of predictiveness over previously known tests. The present invention describes a system of screening for pre-eclampsia, in which a single risk estimate is derived from measurements carried out on biochemical samples obtained during pregnancy.

According to a first aspect of the invention there is provided a method of predicting the risk of pre-eclampsia in a pregnant woman, the method comprising the steps of:

(a) obtaining a sample of blood from the woman;

(b) subsequently assaying the sample for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample; and

(c) determining the risk of pre-eclampsia using the measured levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

Methods according to the present invention are carried out *ex vivo*. In the step (a), the sample of blood may be collected by any suitable means from the pregnant woman. The species free β-human chorionic gonadotrophin (free β-hCG) is distinct from the intact or total form of the molecule which is referred to simply as hCG or total hCG. The assay of the sample in step (b) for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample may be carried out using standard protocols e.g. those based on ELISA (Enzyme-Linked ImmnoSorbent Assay) or RIA (RadioImmunoAssay) , or commercially available kits, e.g. free β-human chorionic gonadotrophin (free β-hCG) can be measured using the solid phase, two site fluoroimmunometric assay based on a direct sandwich technique as described by Stevenson *et al* (*Ann. Clin. Biochem.* **30** 99-100 (1993)) and Spencer *et al* (*Ann. Clin. Biochem.* **29** 506-518 (1992)). Inhibin A can be measured according to the solid phase sandwich ELISA assay described by Groome *et al* (*J. Immunol. Methods* **165** 167-176 (1993); *Clin. Endocrinol.*. **40** 717-723 (1994)). In particular embodiments of the present invention, the assay may also comprise an analysis of the levels of unconjugated oestriol (uE_3), which can be measured using the solid phase, time resolved fluoroimmunoassay described in US-A-4565790 and US-A-4808541. Additionally, since free β-hCG and total hCG are known to be highly correlated in pregnancy, total hCG may also be used as a screening marker for pre-eclampsia in a method according to the present invention as an alternative to free β-hCG. The intact hCG molecule (total

hCG) can be measured directly using exactly the same method as for the free β-subunit with AFP, i.e. sold phase, two-site fluoroimmunometric assay based on a direct sandwich technique. Preferably, the markers used are free β-hCG and Inhibin A measured after 20 weeks of pregnancy, and particularly at the end of the second trimester and the beginning of the third trimester.

In step (c), the determination of the risk of pre-eclampsia can be undertaken by comparing the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample with those in a control sample, or the median level in a group of control samples, i.e. samples from unaffected pregnancies, to provide a prediction of the probability of the onset of pre-eclampsia in the woman. The determination of risk may comprise deriving the likelihood ratio using a multivariate analysis based on distribution parameters from a set of reference data.

Calculation of risk from the measured marker levels is based on the observed relative frequency distribution of marker levels in (a) pre-eclamptic and (b) unaffected pregnancies. Any of the known statistical techniques may be used. Preferably the multivariate Gaussian model is used, which is appropriate where the observed distributions are reasonably Gaussian. Such multivariate Gaussian analysis is in itself known, for example from Wald NJ, Cuckle HS, Densem JW, et al (1988); Maternal serum screening for Down's syndrome in early pregnancy. BMJ 297, 883-887 and Royston P, Thompson SG (1992); Model-based screening by risk with application to Down's syndrome. Stat Med 11, 256-268.

In a preferred method, two Gaussian heights are calculated, (a) one for the pre-eclamptic distribution and (b) the other for the unaffected distribution. For this calculation, the necessary statistical parameters which specify the Gaussian distributions are the mean, the standard deviation and the correlations for the two distributions. The distributions are stored as reference data for use in analysis. The ratio of the two Gaussian heights

gives the likelihood ratio which is a measure of the increased risk of having a disorder, given a particular combination of test results, compared to the background risk, i.e. the risk in the absence of test results.

The estimation of risk consists of multiplying the likelihood ratio by the background risk for pre-eclampsia. The estimated risk is classified as screen-positive or screen negative based on a comparison with a predetermined risk cut-off. The value of the risk cut-off may be altered to vary the detection rate and false positive rate.

Methods in accordance with the present invention may further comprise the step (d) of re-expressing each measured screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman. The screening marker levels may also be adjusted to allow for one or more factors selected from the group of maternal race, maternal weight, multiple birth and diabetic status.

According to a second aspect of the present invention there is provided an apparatus for determining whether a pregnant woman is at an increased risk of pre-eclampsia, the apparatus comprising:

(a) data input means for inputting a measurement of the serum levels of Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) in a sample obtained from said pregnant woman; and

(b) calculation means for determining the risk of pre-eclampsia using the input levels of the serum markers Inhibin A and free β-human chorionic gonadotrophin (free β-hCG).

In certain embodiments of the invention, the calculation means may be arranged to

determine the risk of pre-eclampsia by deriving the likelihood ratio for pre-eclampsia using a multivariate analysis based on distribution parameters derived from a set of reference data. Preferably the multivariate analysis is a multivariate Gaussian analysis.

Apparatus in accordance with this aspect of the invention may further comprise (c) means for re-expressing the levels of each input screening marker Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant women and supplying the re-expressed screening marker levels to said calculation means.

According to a third aspect of the present invention there is provided a method of operating an apparatus as described in accordance with the second aspect to determine the risk of pre-eclampsia in a pregnant woman. The data input means may be used to enter items of data identified as the concentrations of serum markers Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) in a sample obtained from a pregnant woman. The calculation means may be used to calculate the risk of pre-eclampsia using the input levels of the serum markers. The operation the different steps and preferred features are as described above. In another preferred embodiment of this aspect of the invention, the method comprises the steps described in Figures 4, 5, 6 and 7.

According to a fourth aspect of the invention there is provided a kit for predicting the onset of pre-eclampsia in a pregnant woman, comprising means for assaying a sample from the women for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

Preferred features for the second and subsequent aspects of the invention are as for the first aspect *mutatis mutandis*.

The invention will now be further described by way of reference to the following Examples and Figures which are provided for the purposes of illustration only and are not to be construed as being limiting on the invention. Reference is made to a number of Figures in which:

FIGURE 1 shows a probability plot of the Inhibin levels in maternal serum in pre-eclampsia pregnancies (n=23) and unaffected pregnancies (n=96) collected before the onset of proteinuria. MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

FIGURE 2 shows a probability plot of the free β-hCG levels in maternal serum in pre-eclampsia pregnancies (n=22) and unaffected pregnancies (n=93). MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

FIGURE 3 shows a probability plot of the oestriol (uE_3) levels in maternal serum in pre-eclampsia pregnancies (n=13) and unaffected pregnancies (n=66). MoM = multiples of the normal median for unaffected pregnancies of the same gestational age.

FIGURE 4 shows a flowchart illustrating a screening method for pre-eclampsia that involves deriving a risk estimate from measurements made on biochemical samples collected during pregnancy.

FIGURE 5 shows a flowchart illustrating the procedure for calculating multiples of the median (MoM) for biochemical markers. LMP = last menstrual period.

FIGURE 6 shows a flowchart illustrating the procedure for adjusting MoM values to allow for various factors, other than gestational age, that may affect

biochemical marker levels.

FIGURE 7 shows a flowchart illustrating the procedure for selecting the appropriate parameters of the distributions of screening markers in affected and unaffected pregnancies. LMP = last menstrual period.

Example 1: Serum analysis

Serum analysis was carried out on serum collected between 1973 and 1975 from the John Radcliffe Maternity Hospital, Oxford, United Kingdom. Pre-eclampsia was defined as (i) a rise in systolic and diastolic pressure during pregnancy of 30 and 20mm of mercury respectively, compared with the level found at the first antenatal booking visit; (ii) proteinuria greater than 10mg % in a mid-stream urine sample; (iii) renal impairment as judged by the elevation of plasma uric acid levels of 6mg % or more. Nineteen women had blood samples taken after 12 weeks' gestation stored at -40°C. Nine women had one sample, seven had two samples, and three had three samples.

For each sample, three controls were identified selected at random from the patients attending the hospital who had provided a blood sample at the same gestational age in the same calendar quarter and were the same age. Neither cases nor controls were associated with Down's Syndrome or neural tube defects. Serum alphafetoprotein (AFP) and free β-human chorionic gonadotrophin (hCG) were measured using the Wallac-Delfia™ kit, unconjugated oestriol (uE_3) using the Ortho Clinical Diagnostics kit, and Inhibin A using the assay kit produced by Serotec. One sample was sufficient only to measure Inhibin A. For each serum marker, the logs of the medians for the controls were plotted by gestational age and a regression line fitted. The predicted marker values for each gestational age were estimated. All markers were expressed as multiples of their predicted median values for the controls, i.e. MoM's.

All analyses were also completed by using the marker values for each case expressed as

a multiple of the median value of its three controls. This removes the need to model the relationship of the markers with gestational age. The results did not differ significantly from those presented here.

The data were analysed using robust regression with the cluster option in STATA (Stata Corporation, Stata Statistical Software: Release 5.0, College Station, TX (1997)) to take account of repeat samples of some of the women. Table 1 shows the results for the four markers used, classified according to the onset of proteinuria. Inhibin A and free β-hCG values are raised in the pregnancies with pre-eclampsia and the level increases with decreasing time prior to proteinuria and is highest in women after the diagnosis of the disorder. Within three weeks of the onset of proteinuria, the mean Inhibin A value was 3.18 times the median for the controls (95% Confidence Interval - CI, 1.98-5.11), and the mean free β-hCG 3.43 (1.58-7.42). Even 10 weeks prior to the onset of proteinuria these two markers were elevated. The mean uE_3 was significantly reduced in the controls, within three weeks of the onset of proteinuria, MoM = 0.51 (95% CI, 0.42-0.62), but appears to rise again after the onset of proteinuria.

Table 2 shows the observed and expected (using the log Gaussian model) number of affected pregnancies above specified Inhibin A and free β-hCG levels. The correspondence is good. Based on multivariate Gaussian model using the parameters in Table 3 (based on results prior to the onset of proteinuria) in combination they yield an approximately 40% detection rate for a 5% false-positive rate as shown in Tables 5a and 5b.

Using the parameters in Table 4 (based on serum samples collected at 20 weeks gestation or later and before the onset of proteinuria) the detection rate for a 5% false-positive rate is 57% using free β-hCG and Inhibin A, or 67% using free β-hCG, Inhibin A and uE_3, as shown in Tables 5c and 5d.

The reduction in uE_3 needs to be investigated in further studies. These estimates are tentative because they are based on small numbers but provide an indication of the potential use of Down's Syndrome screening markers in the prediction of pre-eclampsia. It provides the opportunity to undertake randomised prevention trials in women at high risk of pre-eclampsia identified at the time of screening for Down's Syndrome, or later in pregnancy.

The results show that Inhibin A and free β-hCG are useful second trimester serum markers for pre-eclampsia. Each provided some independent predictive information because they were not totally correlated. Figures 1 and 2, and Table 2 demonstrate that both the Inhibin A and free β-hCG data fit log Gaussian distributions reasonably well.

The parameters in Tables 3 and 4 referred to above in Example 1 are calculated as follows. The mean $\log_{10}$ MoM in affected pregnancies is estimated from the $\log_{10}$ median value for each marker in affected pregnancies. The median MoM in unaffected pregnancies is 1.0 by definition, and so the $\log_{10}$ MoM value is 0. Standard deviations in affected and unaffected pregnancies are estimated from the slope of the regression lines fitted to the data in Figures 1, 2 and 3, between the 10^{th}-90^{th} centile range. Correlation coefficients between the markers in affected and unaffected pregnancies are estimated from the covariance between markers (after excluding values greater than 3.5 standard deviations from the mean), divided by the product of the standard deviations of the individual markers.

Example 2: Calculation of risk from measured marker levels

Most screening marker levels are known to vary with gestational age. To take account of this variation, each marker level may be expressed as a multiple of the median level (MoM) for unaffected pregnancies of the same gestational age. MoMs may be adjusted in a known way to take account of factors which are known to affect marker levels, such as maternal weight, ethnic group, diabetic status and the number of fetuses carried.

Calculation of risk from the measured marker levels is based on the observed relative frequency distribution of marker levels in (a) pre-eclamptic and (b) unaffected pregnancies. Any of the known statistical techniques may be used. Preferably the multivariate Gaussian model is used, which is appropriate where the observed distributions are reasonably Gaussian. Such multivariate Gaussian analysis is in itself known, for example from Wald NJ, Cuckle HS, Densem JW, et al (1988); Maternal serum screening for Down's syndrome in early pregnancy. BMJ 297, 883-887 and Royston P, Thompson SG (1992); Model-based screening by risk with application to Down's syndrome. Stat Med 11, 256-268. Thus no detailed discussion is necessary, but a summary is given as follows.

If a matrix representation is used, the height H of the Gaussian distribution for a given set of measured levels is given by the equation:

$$H = \frac{1}{\prod(\sigma)\cdot(2\pi)^{p/2}\cdot\det(\mathbf{R})^{1/2}}\exp\left(-\tfrac{1}{2}\cdot\mathbf{Z}^{T}\cdot\mathbf{R}^{-1}\cdot\mathbf{Z}\right)$$

where p is the number of markers, $\Pi(\sigma)$ is the product of the standard deviations for each distribution, $\mathbf{Z}$ is a matrix containing the measured level of each marker expressed in standard deviation units, namely ((measured level - mean) / standard deviation), and $\mathbf{R}$ is a matrix containing the correlations between the screening markers.

Two Gaussian heights are calculated, (a) one for the pre-eclamptic distribution and (b) the other for the unaffected distribution. For this calculation the necessary statistical parameters which specify the Gaussian distributions are the mean, standard deviation and correlations for the two distributions, as summarised in Table 4 (an improved set of parameters compared to the parameters given in Table 3) below for the preferred markers. The distribution parameters are stored as reference data for use in the analysis.

The ratio of the two Gaussian heights gives the likelihood ratio. The likelihood ratio is a measure of the increased risk of having a disorder, given a particular combination of test results, compared to the background risk (that is, the risk in the absence of the test results).

The likelihood ratio is multiplied by the known background risk, to calculate the improved estimate of risk. The estimated risk is classified as screen-positive or screen-negative based on a comparison with a predetermined risk cut-off. The value of the risk cut-off may be altered to vary the detection rate and false-positive rate.

Expected pre-eclampsia detection rates and false-positive rates using the present invention have been estimated using the method previously described in Wald NJ, Cuckle HS, Densem JW, et al (1988) referred to above. Tables 5a and 5b show the performance of screening for pre-eclampsia before the onset of proteinuria, using free β-hCG and Inhibin-A, in terms of the detection rate achieved at specified false-positive rates, and the false-positive rate required to achieve specified detection rates. Tables 5c and 5d show the performance of screening for pre-eclampsia at 20 weeks gestation or later, and before the onset of proteinuria, in the same terms.

Example 3: Computer algorithms for risk calculation

Figures 4 to 7 are flowcharts illustrating a specific method according to the present invention which is explained in detail below.

In the second trimester of pregnancy at around 14 to 22 weeks, a blood sample is drawn in step 1. Subsequently in step 2, the sample is assayed for the biochemical markers selected.

The processing of the measurements taken in step 2 is described below, and may be automated by implementing it in hardware or software.

Data input means are used to input the concentrations (levels) of the biochemical markers in step 3. In step 4, each marker level is re-expressed as a multiple of the median (MoM) level for unaffected pregnancies of the same gestational age and output as data item 5. Step 4 is illustrated in more detail in Figure 5. Stored data LMP 18 and scan 19 specific to respective methods of estimating gestational age are used to select an equation based on stored data which estimates the expected median concentrations for each marker at different gestational ages in step 20. Data LMP 18 is specific to estimation of gestational age based on the first day of the last menstrual period. Data scan 19 is specific to estimation of gestational age from an ultrasound measure of the fetus, usually a biparietal diameter (BPD) or crown-rump length (CRL) measurement. Based on an input in step 21 of the gestational age at the date of sample, for each marker in step 22 the expected median level in unaffected pregnancies of the same gestational age is calculated using the equation selected in step 20. In step 24 each marker level input in step 3 is divided by the expected median for that marker to output the MoM as data item 5.

Optionally the MoMs 5 for the biochemical markers may be adjusted in step 6 which is illustrated in detail in Figure 6. Based on an input of any one or more of maternal weight, ethnic group, diabetic status and the number of fetuses in steps 25 to 28, respectively, stored weight adjustment equations 29, ethnic group adjustments 30, diabetes correction factors 31, and multiple birth correction factors 32 are used in step 33 to adjust the MoMs 5. The adjusted MoMs are output as data item 7.

In step 8, a multivariate Gaussian analysis of the MoMs is performed. For use in this analysis, distribution parameters 10 are selected in step 9 which is described in more detail in Figure 7. For each marker the distribution parameters are stored as reference data 34 to 37 for different methods of estimating gestational age (LMP or scan) and based on whether or not the MoM has been adjusted for maternal weight. In step 38 the appropriate distribution parameters are selected and output as data item 10.

The multivariate Gaussian analysis 8 outputs a likelihood ratio as data item 11. In step 12 the likelihood ratio is multiplied by the stored background risk of pre-eclampsia 13 to output the estimated risk of pre-eclampsia as data item 14. The estimated risk 14 is compared with a predetermined cut-off in step 15 to produce a screen-positive result 16 when the risk is equal to or greater than the cut-off, or a screen-negative result 17 otherwise.

Table 1

Specified serum markers in pregnancies with pre-eclampsia according to timing of collection of serum samples relative to onset of proteinuria

Collection of serum sample relative to onset of proteinuria	Median gestation of onset of proteinuria	No. of women	No. of samples	Median gestation of serum samples	Geometric Mean (MoM) values (95% CI)			
					Inhibin A	AFP	Free β-hCG	uE_3
Over 11 weeks before	29.9	10	10	12.1	1.00 (0.75-1.32)	0.82 (0.61-1.11)	1.29 (1.95-1.76)	0.96 (0.48-1.94)
10-4 weeks before	29.4	6	6	21.5	1.26 (0.66-2.41)	1.13 (0.78-1.64)	2.09 (1.24-3.54)	0.87 (0.67-1.14)
3-0 weeks before*	28.9	6	7†	27.9	3.18 (1.98-5.11)	1.60 (0.58-4.42)	3.43 (1.58-7.42)	0.51 (0.42-0.62)
Up to 3 weeks after proteinuria*	29.9	5	9	32.3	6.66 (3.80-11.68)	1.36 (0.63-2.95)	3.98 (2.52-6.31)	0.93 (0.66-1.29)
Total*	29.9	19	32†	23.4	2.27 (1.52-3.38)	1.14 (0.83-1.57)	2.34 (1.66-3.28)	0.82 (0.60-1.12)
Total prior to onset proteinuria*	29.8	16	23†	21.1	1.49 (1.03-2.16)	1.07 (0.76-1.52)	1.92 (1.28-2.89)	0.78 (0.54-1.13)

MoM - multiples of the median

* - Standard errors adjusted for more than one sample from some women

† - One sample only had measurements of Inhibin

Table 2

Number and percentage of pregnancies with pre-eclampsia collected before onset of proteinuria and unaffected pregnancies according to Inhibin A and free β-hCG

MoM	Inhibin A				Free β-hCG			
	Affected no. (%) (n=23)	Modelled* %	Unaffected no. (%) (n=96)	Modelled* %	Affected no. (%) (n=22)	Modelled* %	Unaffected no. (%) (n=93)	Modelled* %
≥0.5	21 (91%)	93%	87 (91%)	90%	22 (100%)	97%	79 (85%)	84%
≥1.0	17 (74%)	70%	42 (44%)	50%	18 (82%)	82%	46 (49%)	50%
≥1.5	11 (48%)	50%	18 (19%)	22%	13 (59%)	63%	29 (31%)	28%
≥2.0	6 (26%)	35%	13 (14%)	9%	9 (41%)	48%	17 (18%)	16%
≥2.5	6 (26%)	25%	5 (5%)	4%	7 (32%)	36%	7 (8%)	9%
≥3.0	4 (17%)	18%	3 (3%)	2%	7 (32%)	27%	5 (5%)	5%

* - These percentages are estimated assuming both Inhibin A and free β-hCG have log normal distributions

Table 3

Distribution parameters of Inhibin A and free β-hCG in pregnancies with and without pre-eclampsia based on samples collected before onset of proteinuria (23 cases and 96 control samples)

		Log_{10} MoM	
		Inhibin A	Free β-hCG
Means	Unaffected	0	0
	Affected	0.164	0.284
Standard deviations	Unaffected	0.234	0.297
	Affected prior to onset of proteinuria	0.332	0.317
Correlation	Unaffected	0.198	
	Affected	0.899	

Table 4

Standard deviations, correlation coefficients, and means (log_{10} MoM) for selected screening markers in pregnancies with and without pre-eclampsia (affected and unaffected respectively); serum samples collected at 20 weeks gestation or later and prior to the onset of proteinuria (13 cases and 69 control samples)

	Inhibin A	Free β-hCG	uE_3
MEANS			
Free β-hCG	0	0	0
Inhibin-A	0.312	0.389	-0.164
STANDARD DEVIATIONS			
Unaffected	0.217	0.304	0.138
Affected	0.282	0.339	0.200
CORRELATION COEFFICIENTS			
Unaffected			
Free β-hCG	0.276	0.206	
uE3	0.037		
Affected			
Free β-hCG	0.893	-0.508	
uE3	-0.469		

Table 5a

Performance of pre-eclampsia screening using free β-hCG and Inhibin-A before onset of proteinuria: detection rates according to specified false-positive rates

False positive rate (%)	Detection rate (%).
3	36
4	40
5	43
6	45
7	48
8	50

Table 5b

Performance of pre-eclampsia screening using free β-hCG and Inhibin-A before onset of proteinuria: false-positive rates according to specified detection rates

Detection rate (%)	False-positive rate (%).
30	1.8
40	4.2
50	8.2
60	14

Table 5c

Performance of pre-eclampsia screening using free β-hCG, Inhibin A and uE3 measured in serum samples collected at 20 weeks gestation or later and before the onset of proteinuria: detection rates according to specified false-positive rates

False positive rate (%)	Detection rate (%)	
	Free β-hCG and Inhibin A	Free β-hCG, Inhibin A and uE_3
3	50	62
4	54	64
5	57	67
6	60	69
7	62	70
8	64	72

Table 5d

Performance of pre-eclampsia screening using free β-hCG, Inhibin A and uE3 measured in serum samples collected at 20 weeks gestation or later and before the onset of proteinuria: detection rates according to specified false-positive rates

Detection rate (%)	False-positive rate (%)	
	Free β-hCG and Inhibin A	Free β-hCG, Inhibin A and uE_3
30	0.4	0.04
40	1.2	0.2
50	2.9	0.8
60	6.0	2.5
70	12	6.9
80	22	16

CLAIMS

1. A method of predicting the risk of pre-eclampsia in a pregnant woman, the method comprising the steps of:

(a) obtaining a sample of blood from the woman;

(b) subsequently assaying the sample for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample; and

(c) determining the risk of pre-eclampsia using the measure levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

2. A method as claimed in claim 1 which further comprises assaying the sample for the levels of unconjugated oestriol (uE_3).

3. A method as claimed in claim 1 or claim 2 in which the method is carried out after 20 weeks of pregnancy,

4. A method as claimed in claim 3 in which the method is carried out at the end of the second trimester and the beginning of the third trimester.

5. A method as claimed in any one of claims 1 to 4 in which the determination of risk in step (c), is undertaken by comparing the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample with those in a control sample.

6. A method as claimed in claim 5, in which the determination of risk comprises deriving the likelihood ratio using a multivariate analysis based on distribution parameters from a set of reference data.

7. A method as claimed in claim 6, in which the multivariate analysis is a multivariate Gaussian analysis.

8. A method as claimed in 7, in which the estimation of risk consists of multiplying the likelihood ratio by the background risk for pre-eclampsia.

9. A method as claimed in any one of claims 1 to 8, the method further comprising a step (d) of re-expressing each measured screening marker level as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant woman.

10. A method as claimed in claim 9, in which the screening marker levels are adjusted to allow for one or more factors selected from the group of maternal race, maternal weight, multiple birth and diabetic status.

11. An apparatus for determining whether a pregnant woman is at an increased risk of pre-eclampsia, the apparatus comprising:

(a) data input means for inputting a measurement of the serum levels of Inhibin A and free β-human chorionic gonadotrophin (free β-hCG) in a sample obtained from said pregnant woman; and

(b) calculation means for determining the risk of pre-eclampsia using the input levels of the serum markers Inhibin A and free β-human

chorionic gonadotrophin (free β-hCG).

12. An apparatus as claimed in claim 11, in which the data input means (a) further comprises a data input means for inputting a measurement of the serum levels of unconjugated oestriol (uE_3) in a sample obtained from the pregnant woman.

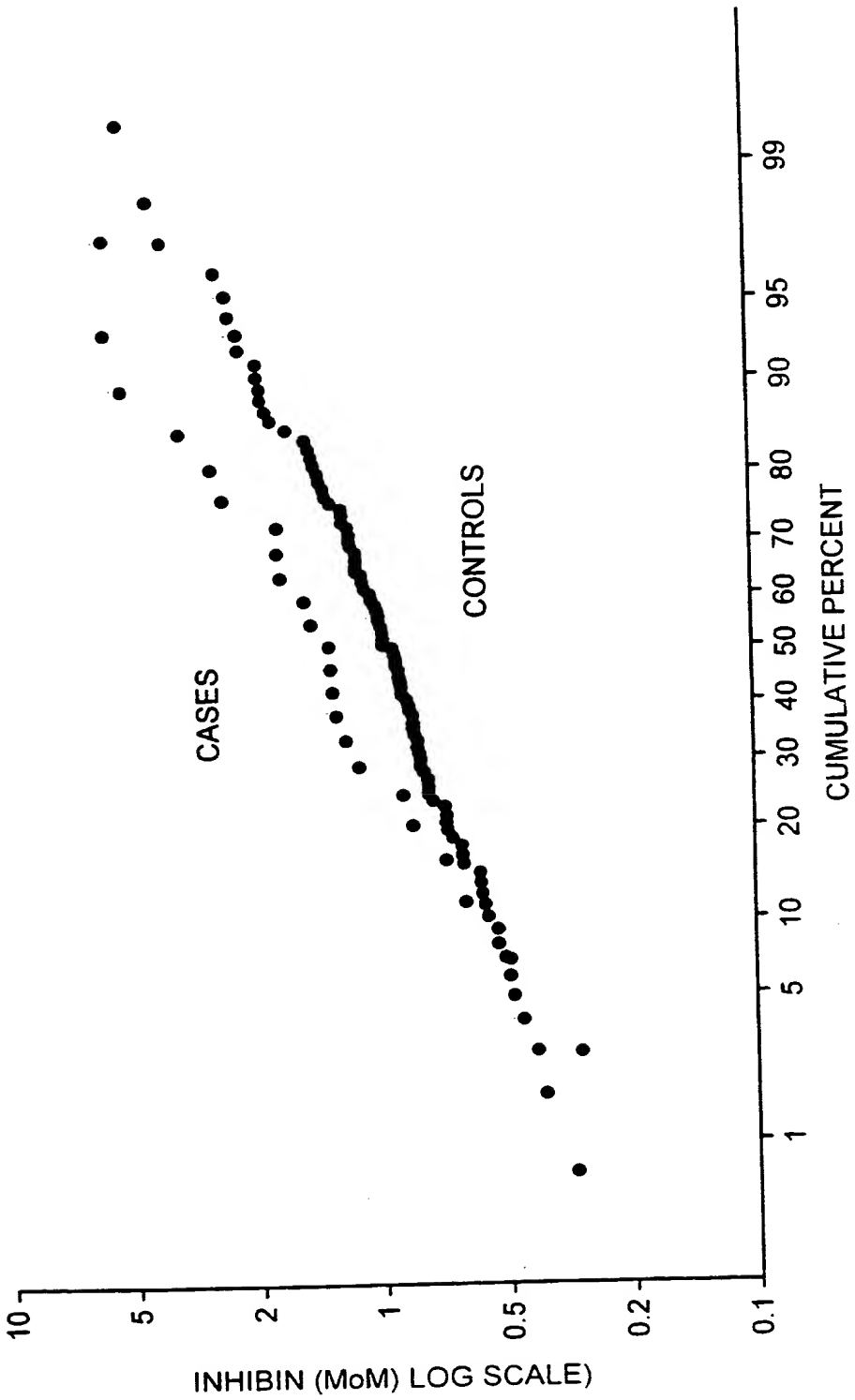
13. An apparatus as claimed in claim 11 or claim 12, in which the calculation means is arranged to determine the risk of pre-eclampsia by deriving the likelihood ratio for pre-eclampsia using a multivariate analysis based on distribution parameters derived from a set of reference data.

14. An apparatus as claimed in claim 13, in which the multivariate analysis is a multivariate Gaussian analysis.

15. An apparatus as claimed in any one of claims 11 to 14, in which the apparatus further comprises (c) means for re-expressing the levels of each input screening marker as a multiple of the median level of the respective screening marker in unaffected pregnancies of the same gestational age as the fetus of the pregnant women and supplying the re-expressed screening marker levels to said calculation means.

16. A kit for predicting the onset of pre-eclampsia in a pregnant woman, comprising means for assaying a sample from the women for the levels of free β-human chorionic gonadotrophin (free β-hCG) and Inhibin A present in the sample.

17. A kit as claimed in claim 16 which further comprises a means for assaying the sample for the levels of unconjugated oestriol (uE_3).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INHIBIN (MoM) LOG SCALE)

FIG. 1

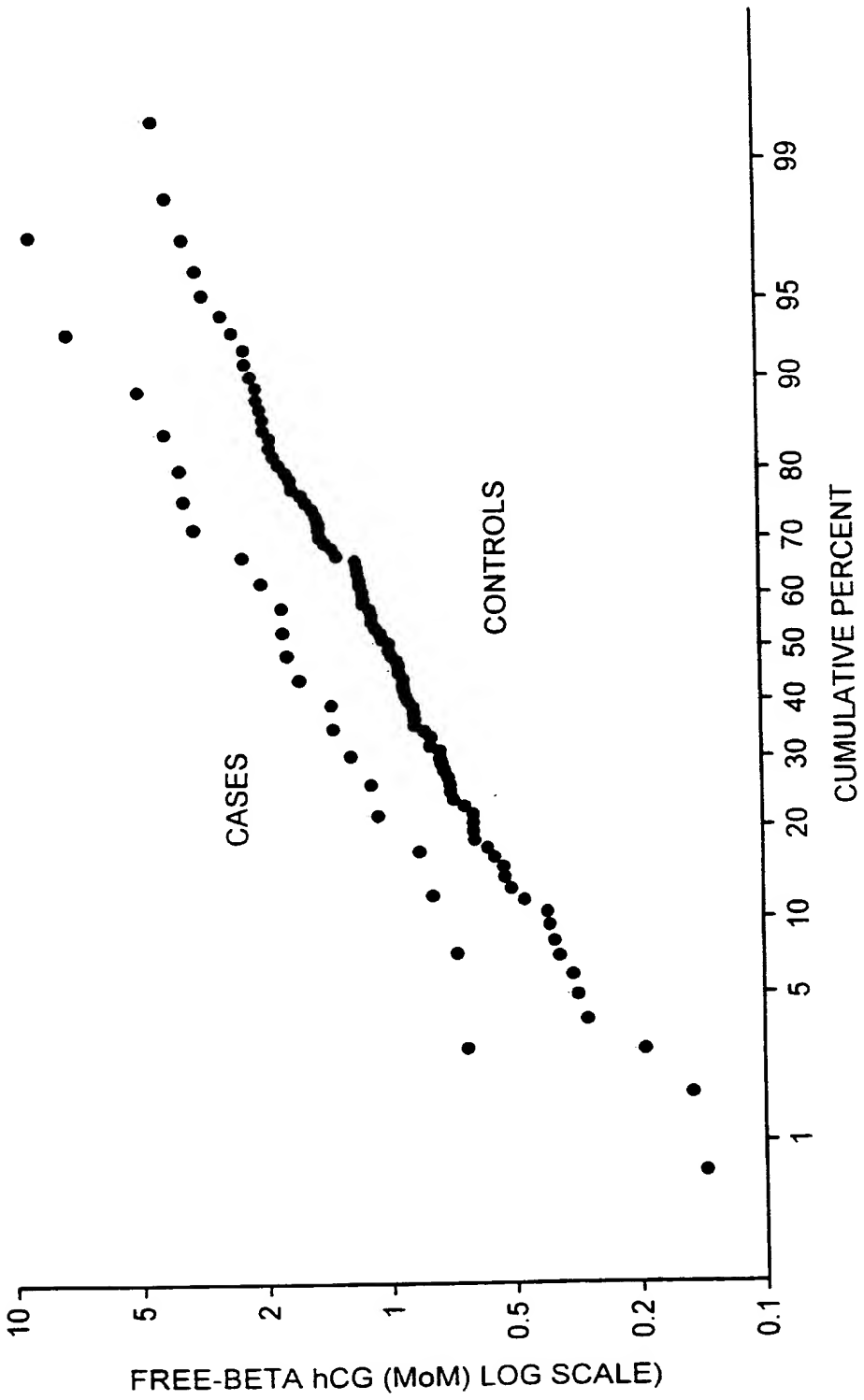
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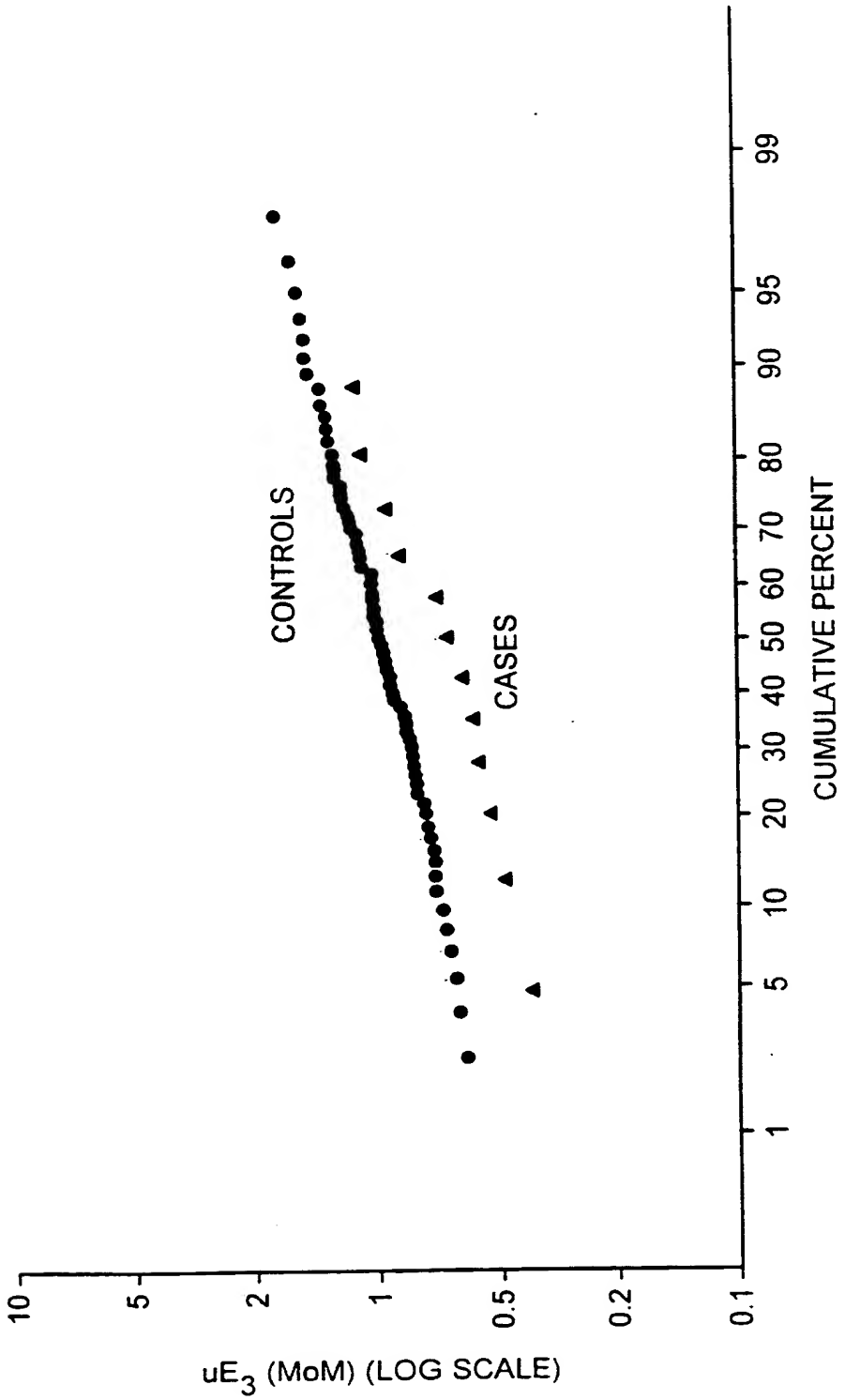
FIG. 2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uE3 (MoM) (LOG SCALE)

FIG. 3

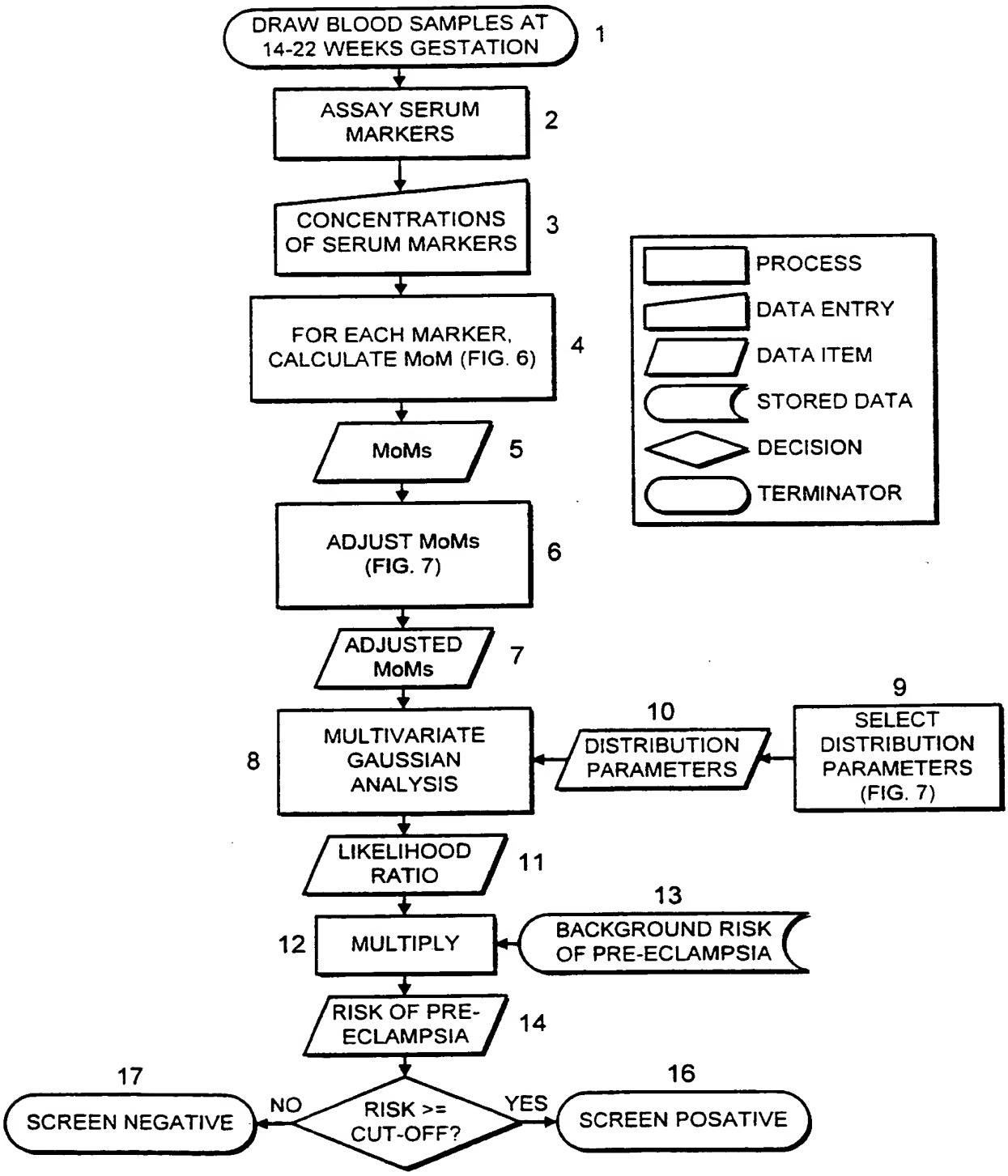
DRAW BLOOD SAMPLES AT 14-22 WEEKS GESTATION
1
ASSAY SERUM MARKERS
2
CONCENTRATIONS OF SERUM MARKERS
3
FOR EACH MARKER, CALCULATE MoM (FIG. 6)
4
MoMs
5
ADJUST MoMs (FIG. 7)
6
ADJUSTED MoMs
7
MULTIVARIATE GAUSSIAN ANALYSIS
8
DISTRIBUTION PARAMETERS
10
SELECT DISTRIBUTION PARAMETERS (FIG. 7)
9
LIKELIHOOD RATIO
11
MULTIPLY
12
BACKGROUND RISK OF PRE-ECLAMPSIA
13
RISK OF PRE-ECLAMPSIA
14
RISK >= CUT-OFF?
NO
YES
SCREEN NEGATIVE
17
SCREEN POSATIVE
16
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 4

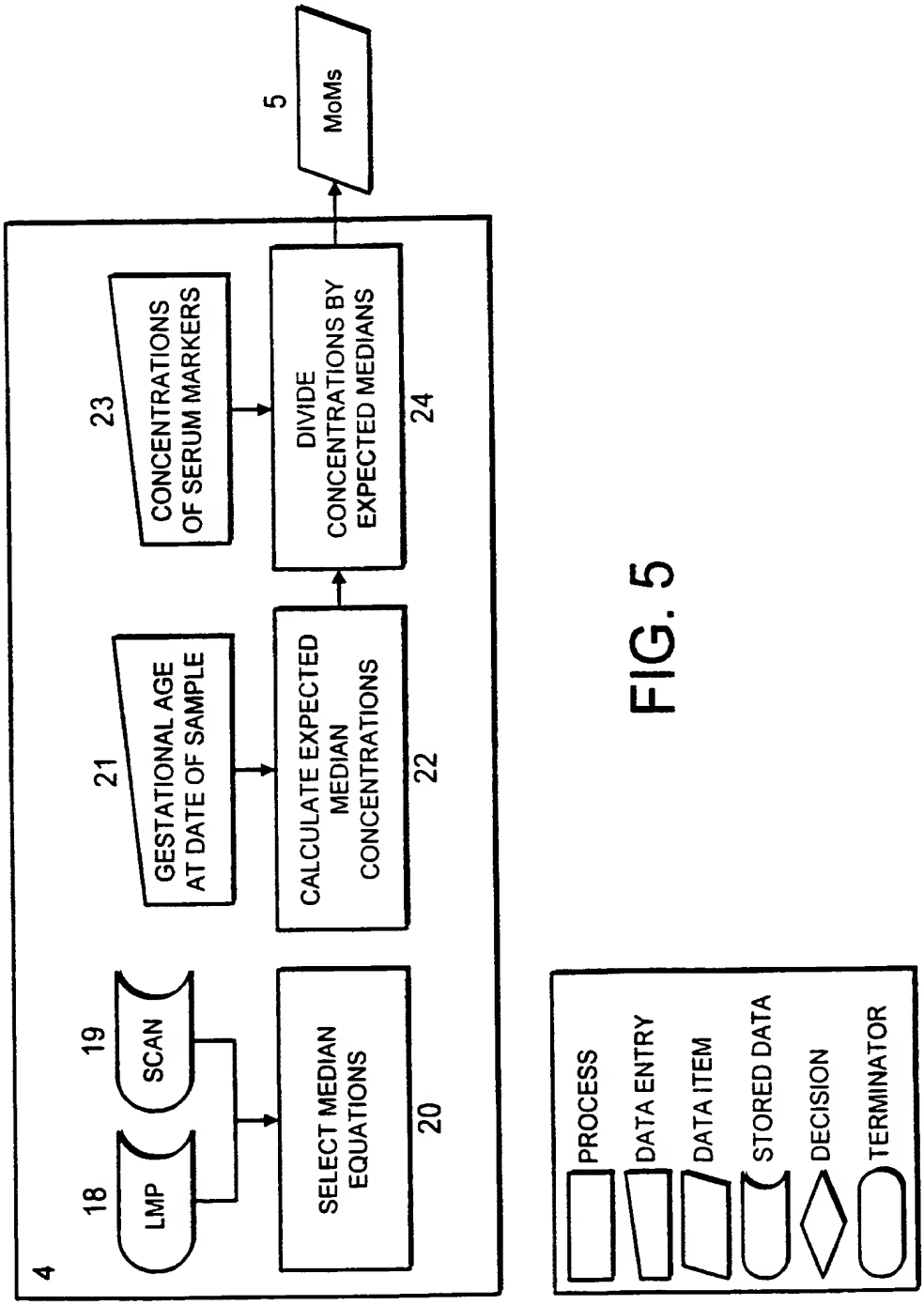
4
18
LMP
19
SCAN
SELECT MEDIAN EQUATIONS
20
21
GESTATIONAL AGE AT DATE OF SAMPLE
CALCULATE EXPECTED MEDIAN CONCENTRATIONS
22
23
CONCENTRATIONS OF SERUM MARKERS
DIVIDE CONCENTRATIONS BY EXPECTED MEDIANS
24
5
MoMs
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 5

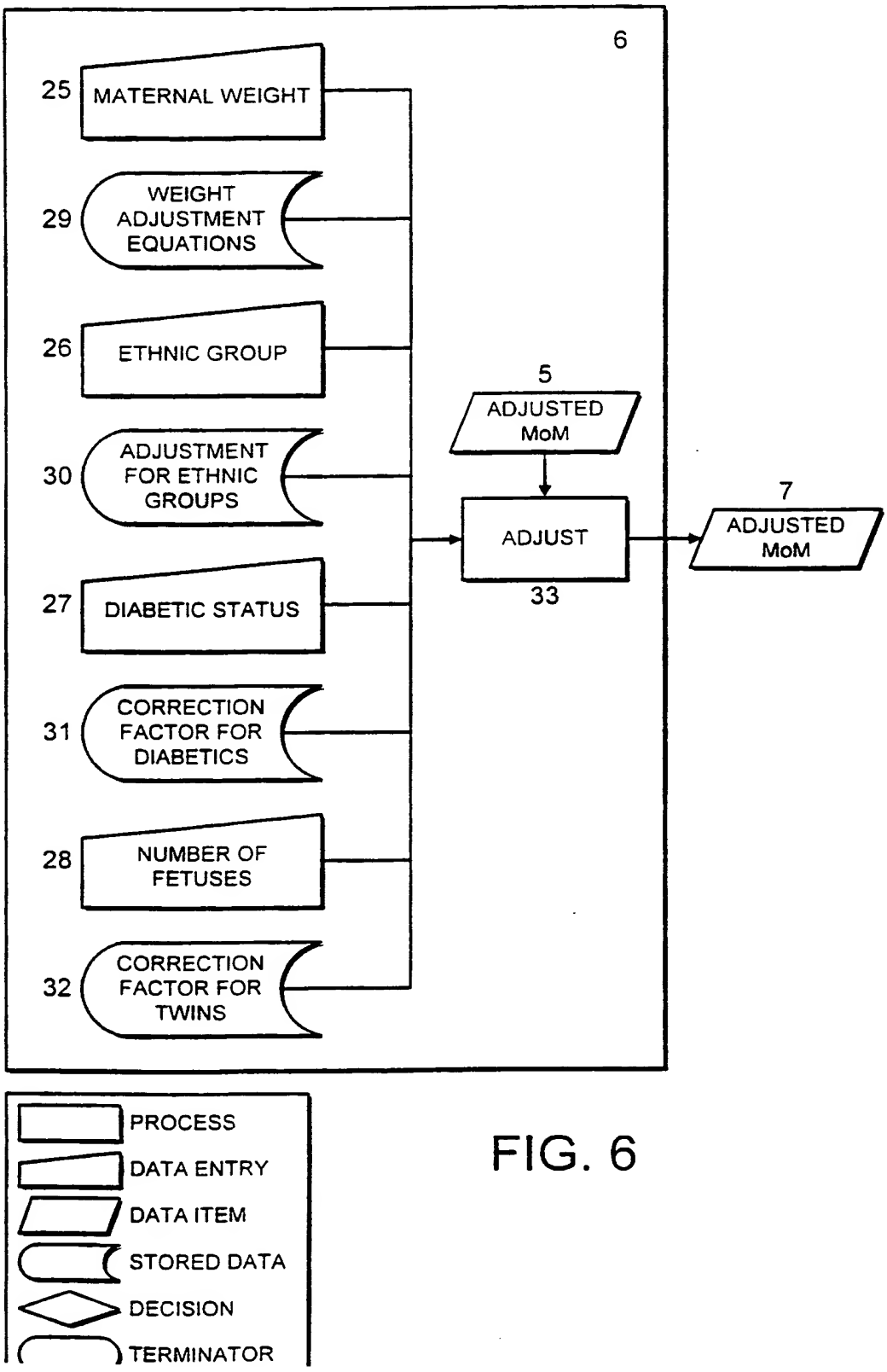
6
25
MATERNAL WEIGHT
29
WEIGHT ADJUSTMENT EQUATIONS
26
ETHNIC GROUP
30
ADJUSTMENT FOR ETHNIC GROUPS
27
DIABETIC STATUS
31
CORRECTION FACTOR FOR DIABETICS
28
NUMBER OF FETUSES
32
CORRECTION FACTOR FOR TWINS
5
ADJUSTED MoM
ADJUST
33
7
ADJUSTED MoM
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 6

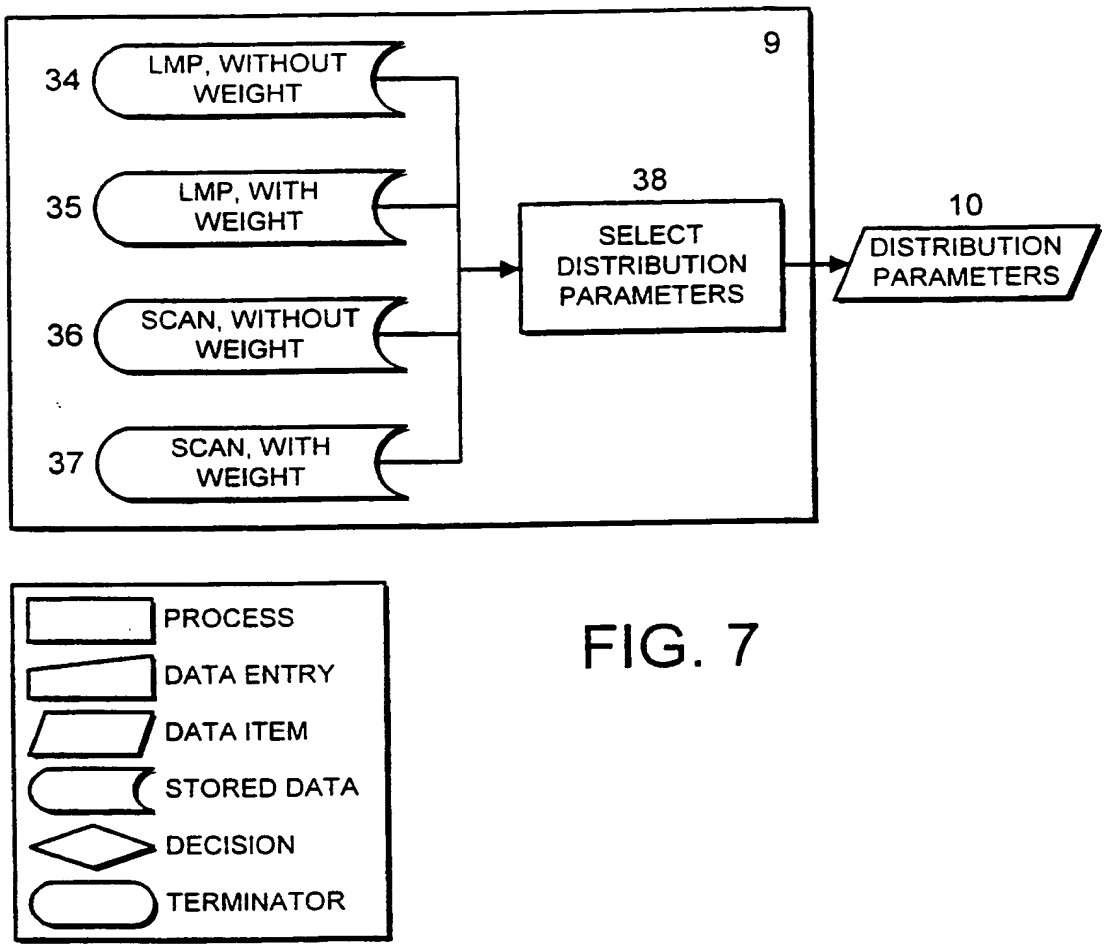
9
34
LMP, WITHOUT WEIGHT
35
LMP, WITH WEIGHT
36
SCAN, WITHOUT WEIGHT
37
SCAN, WITH WEIGHT
38
SELECT DISTRIBUTION PARAMETERS
10
DISTRIBUTION PARAMETERS
PROCESS
DATA ENTRY
DATA ITEM
STORED DATA
DECISION
TERMINATOR

FIG. 7